Chiral Resolution, Pharmacological Characterization, and Receptor Docking of the Noncompetitive mGlu1 Receptor Antagonist (±)-2-Hydroxyimino-1a,2-dihydro-1*H*-7-oxacyclopropa[*b*]naphthalene-7a-carboxylic Acid Ethyl Ester

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Racemic CPCCOEt ((1a*RS*,7a*RS*)-2-hydroxyimino-1a,2-dihydro-1*H*-7-oxacyclopropa[*b*]naphthalene-7a-carboxylic acid ethyl ester, **(±)-1**) derivatives have been shown to be subtype-selective metabotropic glutamate (mGlu) 1 receptor antagonists (Annoura et al. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 763−766). The optical isomers of **(±)-1** have been separated by chromatography on a chiral stationary phase. The absolute configuration at the C-1a and C-7a positions was determined using X-ray crystallography of an amide derivative with the methyl ester of L-phenylalanine (L-PheOMe) (**(+)-6**). In a phosphoinositol (PI) turnover assay at the cloned human mGlu1b receptor, **(−)-1** and the new amide derivatives **(−)-5** and **(−)-6**, all of which have (1a*S*,7a*S*)-stereochemistry on the chromane ring system, showed IC_{50} values of 1.5, 0.43, and 0.93 μM, respectively. In contrast, **(+)-1** and the new amide derivatives **(+)-5** and **(+)-6** were found to be inactive up to a concentration of 30 μM indicating a selectivity for the (−)-enantiomers of at least 70-fold. In a previous study (Litschig et al. *Mol. Pharmacol.* **1999**, *55*, 453−461) we demonstrated using site-directed mutagenesis that the interaction site of **(±)-1** is located in the transmembrane (TM) domain of hmGlu1b. To suggest a plausible binding mode of **(−)-1**, we have built a molecular mechanics model of the putative seven TM domain of hmGlu1 based on the α-carbon template of the TM helices of rhodopsin. A receptor docking hypothesis suggests that the OH of T815 (TMVII) comes in close contact with the oxime OH of **(−)-1** and **(−)-5**, whereas no such close interactions could be demonstrated by docking of **(+)-1**.

Introduction

L-Glutamic acid (L-Glu) is the main excitatory neurotransmitter in the mammalian central nervous system and is thought to play a role in a number of pathophysiological processes such as epilepsy, pain, neurodegeneration, and schizophrenia.[1−3] L-Glu exerts its action via the activation of ionotropic glutamate (iGlu) receptors and metabotropic glutamate (mGlu) receptors. To date mGlu receptors consist of a family of eight different G-protein-coupled receptors (GPCRs) classified into three groups according to their amino acid sequence homologies, second-messenger coupling, and pharmacology.[4] Group I receptors (mGlu1 and -5) couple to phospholipase C, whereas group II (mGlu2 and -3) and group III (mGlu4, -6, -7, and -8) receptors are negatively coupled to adenylate cyclase. Despite differences in primary structures and function, all mGlu receptors are characterized by a large conserved N-terminal extracellular domain that is involved in the selective recognition of agonists and competitive antagonists.

A variety of antagonists at the group I mGlu receptors have been described and can be classified into two groups according to their mode of inhibition. Competitive antagonists such as (*RS*)-3-[1-amino-1-carboxy-2-(9*H*-xanthen-9-yl)ethyl]cyclobutanecarboxylic acid (LY357366)[5] and (+)-2-methyl-4-carboxyphenylglycine (LY367385)[6] are amino acids derivatives which interact at the glutamate binding site located in the large extracellular N-terminal domain (Figure 1).[7,8] Recent advances have elucidated two novel compound classes with a noncompetitive mode of inhibition and no structural analogy to amino acids (Figure 1): the first class of antagonists is exemplified by **(±)-1**[9,10] and is selective for the mGlu1 receptor; the second class is exemplified by 6-methyl-2-phenylazopyridin-3-ol (SIB-1757), 2-methyl-6-((*E*)-styryl)pyridine (SIB-1893),[11] and 2-methyl-6-(phenylethynyl)pyridine (MPEP)[12] and is selective for the mGlu5 receptor.

Using chimeric hmGlu1 and hmGlu5 receptors and site-directed mutagenesis, we previously demonstrated that **(±)-1** interacts with a novel pharmacological site in the transmembrane (TM) domain, clearly separated from the glutamate binding site which is located in the large extracellular N-terminal domain.[13] In addition we showed that the nonconserved amino acid residues T815 and A818, in TM segment VII, are necessary for mediating the antagonist activity of **(±)-1** (Figure 2). Further investigations of the **1**/hmGlu1 receptor interactions using molecular modeling have been hampered due to the chiral nature of **1**. In continuation of our previous work we have now separated the two enantiomers, determined the absolute configuration, and

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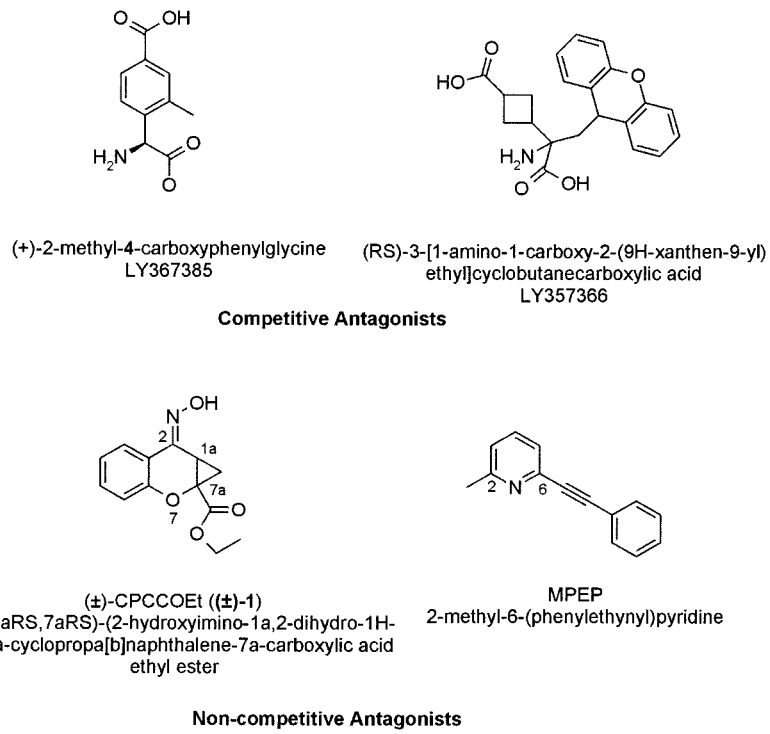
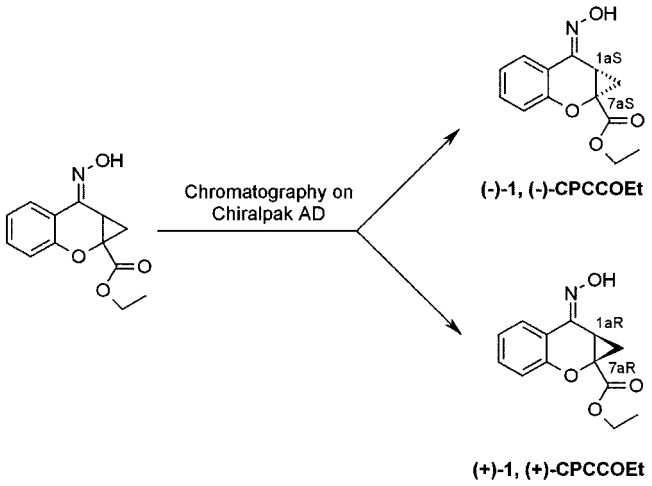


Figure 1. Chemical structures of competitive and noncompetitive group I mGlu receptor antagonists.

Scheme 1. Chromatographic Separation of **(+)-1** and **(−)-1**

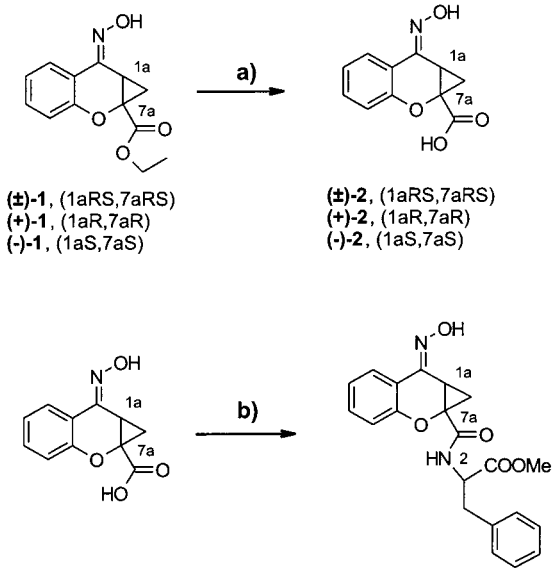


pharmacologically evaluated the two enantiomers as well as six novel amide derivatives. A model of the human mGlu1 receptor TM domain consisting of seven helices was built, and docking experiments with the enantiomers of **(±)-1** were performed to interpret these pharmacological results.

Results

Synthesis. A stereoselective synthesis leading to enantiomerically pure cyclopropanated chromane derivatives has not been described yet. To obtain pure enantiomers, racemic **(±)-1** was synthesized as previously described[9] and the enantiomers were separated by chromatography on a chiral stationary phase using a Chiralpak AD[14,15] column. The (+)- and (−)-enantiomers of **1** were obtained both with very high enantiomeric purity (>99.5%) (Scheme 1). In the case of the amide derivatives with phenylalanine **3** and **4**, a diastereomeric separation might constitute an alternative since a separation was observed on TLC (for details, see Experimental Section).

Scheme 2[a]



[a] Reagents and conditions: (a) NaOH/THF, rt; (b) TBTU, ethyldiisopropylamine, phenylalanine methyl ester hydrochloride, DMF, rt.

The absolute configuration of the cyclopropanated chromane structure has never been determined. A direct determination of the absolute configuration of **1** by X-ray crystallography was not possible due to the absence of

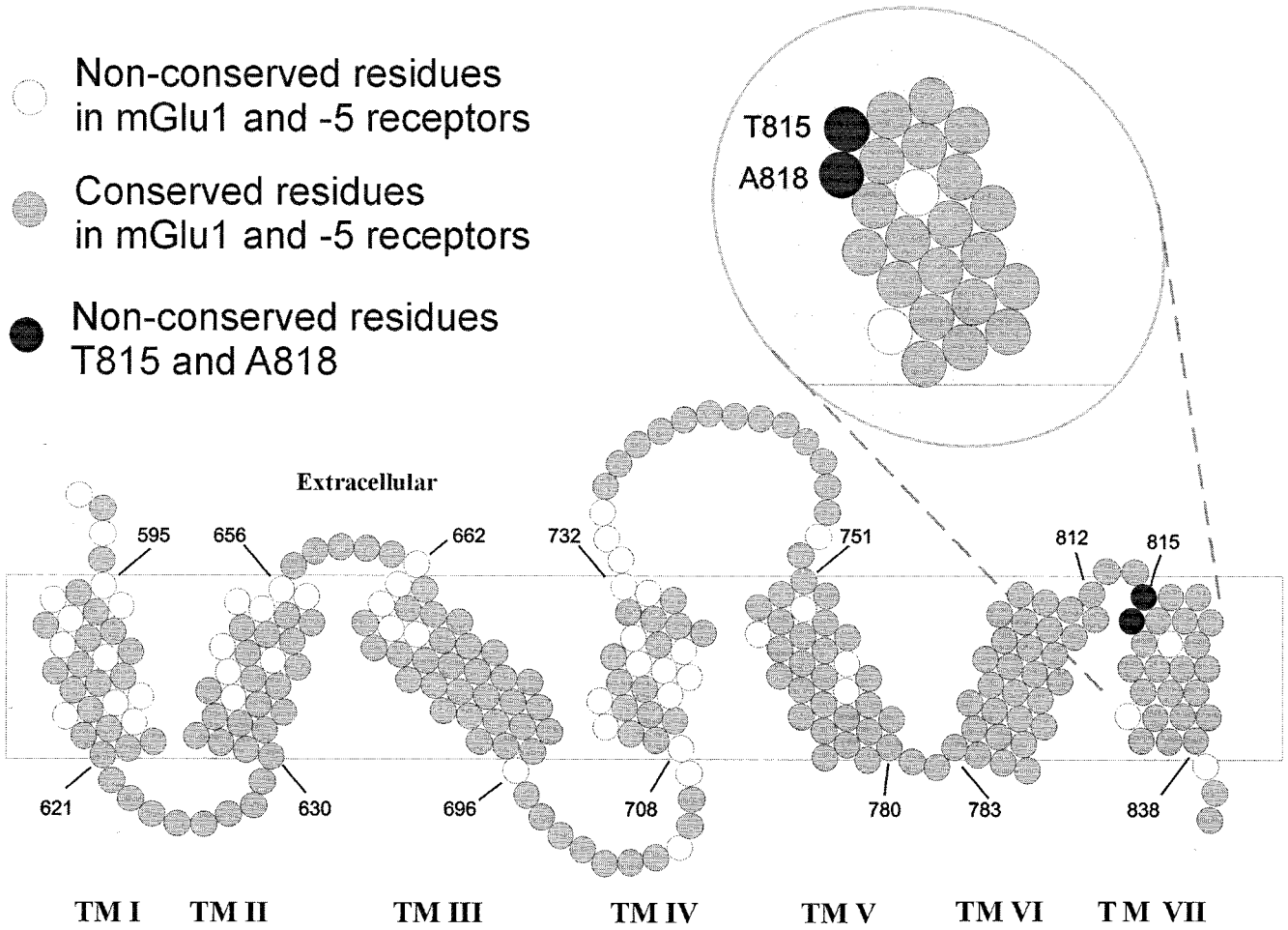


Figure 2. Schematic representation of the seven TM domain of the hmGlu1 receptor. Conserved amino acids between hmGlu1 and hmGlu5 are shown in gray. Nonconserved amino acids between hmGlu1 and hmGlu5 are depicted in white. Mutation sites T815 (TMVII) and A818 (TMVII) which mediate functional inhibition by **(±)-1** are highlighted in black. The glutamate binding site is located in the large N-terminal extracellular domain (not shown).

a heavy atom. Therefore, we have used an indirect method which consists of the coupling of the chiral auxiliary, phenylalanine methyl ester (Phe-OMe), with the carboxyl group of **1** via an amide bond (Scheme 2).

Absolute Structure Determination. The assignment of the configuration at the C-1a and C-7a positions was based on X-ray crystallography of the amide derivative **(+)-6**. The absolute configuration was deduced from the L-configuration of the L-Phe-OMe which was used for the synthesis and was found to be (1a*R*,-7a*R*). The spatial coordinates of the crystal structure are provided as Supporting Information. The heterocyclic six-membered ring adopts a slight boat conformation. The oxime side chain is in a (*E*)-conformation and forms an angle of 9.9° to the plane of the chromane ring system (Figure 3).

In Vitro Pharmacology. The pharmacology of the chromane derivatives was evaluated at the cloned hmGlu1b receptor stably expressed in CHO cells using a phosphoinositol (PI) hydrolysis assay (Figure 4, Table 1).[10] **(±)-1** potently inhibited quisqualate-induced stimulation of PI hydrolysis with an IC_{50} of 3.4 μM (2.0, 5.8; 95% confidence intervals; $N = 4$). The (−)-enantiomer (**(−)-1**) showed an IC_{50} of 1.5 μM (0.3, 8.3; 95% confidence intervals; $N = 3$), whereas the (+)-enantiomer (**(+)-1**) was inactive up to 30 μM. Only at 100 μM an inhibition of 53% was observed, giving an estimated IC_{50}

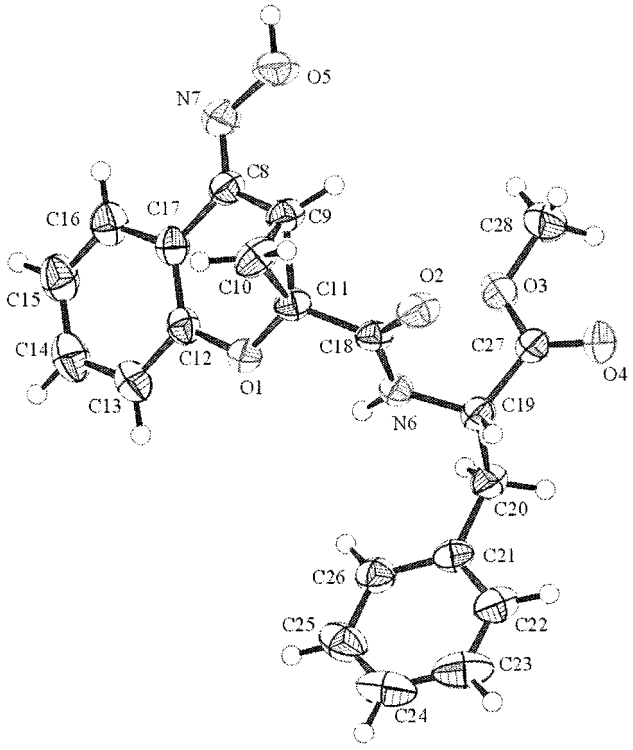


Figure 3. Ortep plot of **(+)-6**.

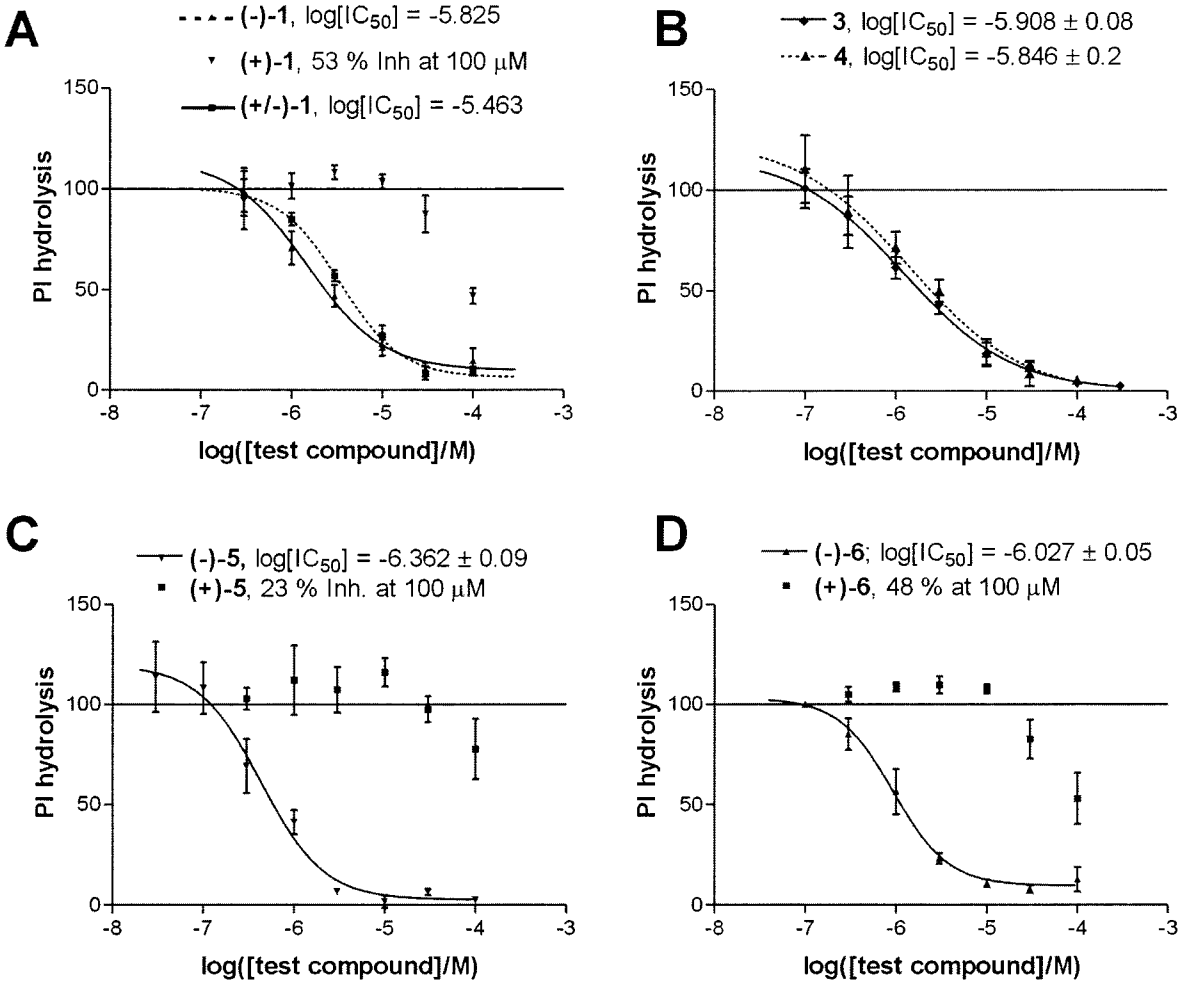
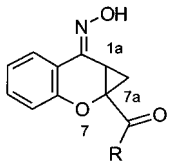


Figure 4. Concentration−response curves for inhibition of quisqualate-induced PI hydrolysis in CHO cells expressing the hmGlu1b receptor. Curves were obtained by fitting the four-parametric logistic equation to the data using Prism2.0 (GraphPad, San Diego, CA). The results presented are the means ± SEM of data from 3 independent experiments performed in triplicate: (A) **(±)-1**, **(+)-1**, **(−)-1**; (B) **3**, **4**; (C) **(+)-5**, **(−)-5**; (D) **(+)-6**, **(−)-6**.

Table 1. Inhibition of Quisqualate-Induced PI Hydrolysis Measured in CHO-hmGlu1 Receptor-Expressing Cells[a]



compd	R	chirality at C-1a and C-7a	IC_{50}, μM (95% CI) or effect at given concentration in parentheses
(±)-1	OEt	(1a*RS*,7a*RS*)	3.4 (2.0, 5.8)
(+)-1	OEt	(1a*R*,7a*R*)	ne (30 μM)
(−)-1	OEt	(1a*S*,7a*S*)	1.5 (0.3, 8.3)
3	*S*-Phe-OMe	(1a*RS*,7a*RS*)	1.2 (0.7, 2.1)
4	*R*-Phe-OMe	(1a*RS*,7a*RS*)	1.4 (0.32, 6.2)
(+)-5	*R*-Phe-OMe	(1a*R*,7a*R*)	ne (30 μM)
(−)-5	*S*-Phe-OMe	(1a*S*,7a*S*)	0.43 (0.24, 0.77)
(+)-6	*S*-Phe-OMe	(1a*R*,7a*R*)	ne (30 μM)
(−)-6	*R*-Phe-OMe	(1a*S*,7a*S*)	0.93 (0.65, 1.3)

[a] IC_{50} values are given in μM with parentheses indicating 95% confidence intervals and were obtained by fitting the data with a four-parametric logistic equation using Prism2.0 (GraphPad, San Diego, CA). For each determination three independent experiments were performed in triplicate; ne = no effect.

of 100 μM and a stereoselectivity factor of at least 70. Higher concentrations of **(+)-1** could not be tested due to limited solubility (Figure 4, Table 1).

Investigation of the amide derivatives revealed **(−)-5** and **(−)-6** as the most potent derivatives with IC_{50} values of 0.43 μM (0.24, 0.77; 95% confidence intervals; $N = 3$) and 0.93 μM (0.65, 1.3; 95% confidence intervals; $N = 3$), respectively. As for **1**, the respective enantiomers **(+)-5** and **(+)-6** were devoid of activity up to 30 μM confirming the importance of the configuration of the cyclopropanated chromane ring. Interestingly, the configuration of the α-carbon of the phenylalanine moiety seemed not to play an important role since the diastereoisomers **3** and **4** were equally potent and **(−)-5** and **(−)-6** differed only 2-fold in their IC_{50} value (Figure 4, Table 1).

Modeling. In a previous study[13] we demonstrated using site-directed mutagenesis the interaction of **(±)-1** with the two amino acids T815 and A818 located close to the extracellular side of TMVII. To suggest a plausible binding mode of **1** to both residues of hmGlu1, we have built a model of the putative seven TM domain of hmGlu1b with the SYBYL[16] software using the α-carbon template of TM helices of bovine rhodopsin[17] (for details, see Experimental Section). The sequence assignment of the seven TM domains is given in Table 2.[18]

To this hmGlu1 receptor model we have manually docked **(−)-1** with the oxime group forming a H-bond to the hydroxyl group of T815 and the chromane system

Table 2. Assignment of α-Helical TM Segments of hmGlu1[a]

TM	S	amino acid sequence	E	Baldwin numbering
I	595	(IESI)IAIAFSCLGILVTLFVTLIF**V**LYRDTP	621	101−127
II	630	LCYIILAGIF**L**GYVCPFTLIAKPTTTS	656	200−226
III	662	LLVGLSSAMCYSALVTKTNRIAR**I**LAGSKKK**I**CTR	696	298−332
IV	708	IIASILISVQLTLVVTLIIMEPPMP	732	401−425
V	751	LGVVAPLGYNGLLIMSCTY**Y**AFKTRNVPAN	780	503−532
VI	783	EAKYIAFTMYTTCIIWLAFVPIYFGSNYKI<IT>	812	596−625
VII	815	TCFAVSLSVTVALGCMFTPKMYII(IA**K**)	838	703−726

[a] Bold letters denote residues highly conserved in GPCRs of the rhodopsin family, and underlined letters (TMVII) denote residues identified as essential for antagonism of CPCCOEt. Residues <IT> have been inserted for loop building. Residues (IESI) and (IAK) have not been used in the receptor model. S = starting amino acid; E = ending amino acid.

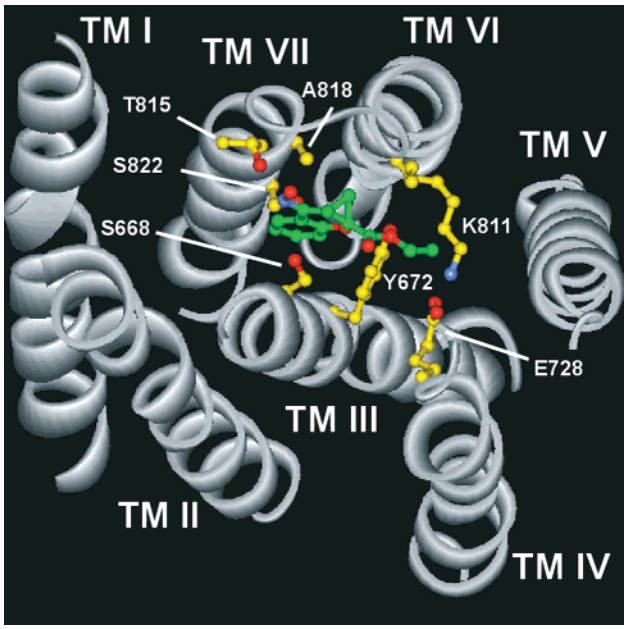


Figure 5. Model of **(−)-1** (shown with green carbon atoms, nitrogens in blue, and oxygens in red) docked to the model of the hmGlu1 receptor and minimized with X-PLOR. Closely interacting amino acid residues are highlighted (carbon atoms in yellow, nitrogens in blue, and oxygens in red) (T815, A818, S822 (TMVII); S668, Y672 (TMIII)). The possible salt bridge between K811 (TMVI/EL3) and E728 (TMIV) is also shown.

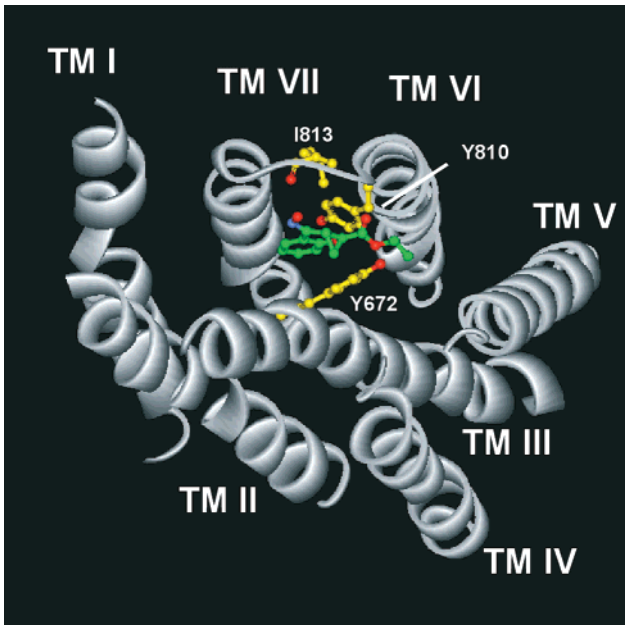


Figure 6. Model of **(+)-1** (shown with green carbon atoms, nitrogens in blue, and oxygens in red) docked to the model of the hmGlu1 receptor and minimized with X-PLOR. Closely interacting amino acid residues are highlighted (carbon atoms in yellow, nitrogens in blue, and oxygens in red) (I813 (EL3); Y810 (TMVI); Y672 (TMIII)).

occupying the TM cavity mainly formed by helices III, VI, and VII. This complex was minimized using an X-PLOR[19] molecular dynamics protocol, and the structure with the lowest energy (E = −1605 kcal/mol, grad(E) = 0.3) was taken as one plausible model of **(−)-1** binding to hmGlu1 (Figure 5). The H-bond between the oxime O and the OH group of T815 is maintained, and an additional H-bond interaction is formed between the chromane O or ester O and the OH of Y672 (TMIII). Hydrophobic contacts are formed between the cyclopropane ring and the methyl group of A818 (TMVII). In analogy to interactions found in crystal structures,[20] the model shows possible contacts between the chromane phenyl moiety and the amino acid residues S668 (TMIII) and S822 (TMVII). A favorable salt bridge is possible between K811 (TMVI/EL3) and E728 (TMIV).

In the same way, **(+)-1** was manually docked to the receptor model and minimized. The structure lowest in energy (E = −1580 kcal/mol, grad(E) = 0.3) was taken to illustrate the weaker fit of **(+)-1** into the TM domain as shown in Figure 6. In this case no involvement of T815 or A818 in the interactions with the ligand was found. Putative H-bonds are formed between the oxime OH and the carbonyl O of I813 (EL3) and between the ester O (not the chromane O) and the OH of Y672 (TMIII). Hydrophobic contacts are found between the chromane ring and Y810 in (TMVI). In addition, this docking did not show the formation of the favorable salt bridge between K811 (TMVI/EL3) and E728 (TMIV).

Using the same protocol we docked the most potent derivative **(−)-5** and its enantiomer **(+)-5**. Optimization of both complexes showed that the best structure obtained with **(−)-5** (E = −1677 kcal/mol, grad(E) = 0.005) (Figure 7) was 26 kcal/mol lower in energy compared to the structure obtained for **(+)-5** (not shown). For the active enantiomer the minimization showed a similar binding mode as for the chromane part of **(−)-1** with H-bonds formed between the oxime H-atom and the OH group of T815 (TMVII) and between the chromane O and the phenolic OH of Y672 (TMIII). In analogy to the model with **(−)-1**, this model suggests possible interactions of the chromane part of **(−)-5** with S668 (TMIII) and S822 (TMVII). Hydrophobic contacts are formed between the phenyl ring of Phe-OMe and the hydrophobic side chain atoms of K811 (TMVI/EL3) and I812 (EL3). The favorable salt bridge between K811 (TMVI/EL3) and E728 (TMIV) found in the model with **(−)-1** (Figure 5) is also formed in this model.

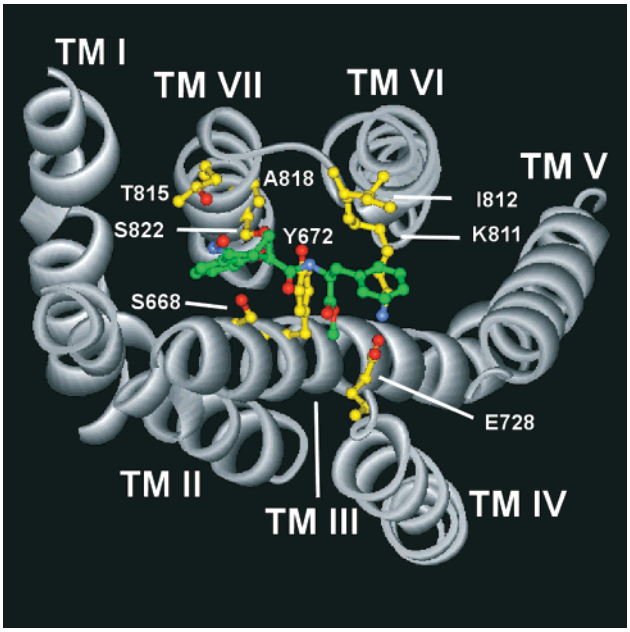


Figure 7. Model of **(−)-5** (shown with green carbon atoms, nitrogens in blue, and and oxygens in red) docked to the model of the hmGlu1 receptor and minimized with X-PLOR. Closely interacting amino acid residues are highlighted (carbon atoms in yellow, nitrogens in blue, and oxygens in red) (T815, A818, S822 (TMVII); S668, Y672 (TMIII); K811, I812 (TMVI/EL3)). The possible salt bridge between K811 (TMVI/EL3) and E728 (TMIV) is also shown.

Discussion

Recently, we showed that **(±)-1** is a selective mGlu1 receptor antagonist that inhibits receptor activity with a noncompetitive mechanism without affecting the binding of agonists to the glutamate binding site located in the large extracellular domain.[13] Site-directed mutagenesis showed that inhibition by **(±)-1** requires the mGlu1-specific residues, T815 and A818 in TM segment VII. Substitution of T815 and A818 of mGlu1 by the corresponding residues of mGlu5 completely eliminated inhibition by **(±)-1**, while substitution of these residues into the mGlu5 receptor conferred **(±)-1** inhibition with equal potency as in the wild-type mGlu5 receptor.[13] The aim of the current study was to investigate the interactions of the enantiomers of **(±)-1** with this allosteric binding site located in the TM domain. The (+)- and (−)-enantiomers of **1** were obtained using a chromatographical separation on chiral phase, and the absolute configuration was determined by X-ray crystallography with the amide derivative **(+)-6**. Pharmacological characterization demonstrated that a (1a*S*,7a*S*)-configuration on the cyclopropanated chromane ring system is necessary for antagonist activity. The (1a*R*,7a*R*)-isomers were devoid of activity up to 30 μM leading to a stereoselectivity factor of at least 70, which is comparable to the stereoselectivity of antagonists of other GPCRs.[21,22] In contrast, no stereoselective recognition was seen for the phenylalanine moiety since the derivatives **3** (L-Phe) and **4** (D-Phe) or **(−)-5** (L-Phe) and **(−)-6** (D-Phe) showed comparable potencies. This suggests that the affinity for this allosteric site is more dependent on the configuration of the cyclopropanated chromane ring system than on the stereochemistry of the phenylalanine moiety.

To suggest a possible binding mode of **1** and its new derivatives, we constructed a model of the mGlu1 TM domain using the structure of bovine rhodopsin as a template.[17] Docking experiments with the active isomers **(−)-1** and **(−)-5** and their inactive counterparts **(+)-1** and **(+)-5** clearly support the above pharmacological data. The active enantiomers show interactions with residues T815 and A818, previously identified by site-directed mutagenesis as the critical determinants for selective mGlu1 receptor inhibition.[13] In particular, the oxime O of **(−)-1** and **(−)-5** can form a H-bond with the OH group of residue T815. In addition, possible lipophilic interactions are formed between the cyclopropyl and phenyl moieties and the methyl group of residue A818. Docking of the inactive enantiomers did not show possible interactions with these critical amino acids.

According to this model the binding site of the CPCCOEt series not only involves residues in TMVII but also residues close to the extracellular surface of TMIII and TMVI. Similarly located binding sites were proposed for members of other GPCR families such as the A_3 adenosine[22] and 5-HT_{2A} serotonin[23] receptors which have no sequence similarity to mGlu receptors. For the adenosine receptor antagonists are thought to bind in a pocket formed by the TM domains III, V, and VI, whereas for the serotonin receptor TM domains III, VI, and VII are involved. This suggests that the novel allosteric binding site for the noncompetitive antagonist **1** at the mGlu1 receptor is homologous to the binding site of antagonists at classical GPCRs.

Experimental Section

Chemical Synthesis. Melting points (uncorrected) were determined using a Büchi 510 apparatus. MS (mass spectra) were recorded with a Micromass Platform II using electron spray ionization (+ or −). ^{1}H and ^{13}C NMR spectroscopy were performed on Varian Gemini-200, -300 or -400 at 300 or 400 MHz for ^{1}H NMR and at 50, 75 or 100 MHz for ^{13}C NMR. Optical rotations were measured on a Perkin-Elmer 241 polarimeter and are reported at 589 nm (Na D-line).

(+)-CPCCOEt, (+)-(1a*R*,7a*R*)-(2-Hydroxyimino-1a,2-dihydro-1*H*-7-oxacyclopropa[*b*]naphthalene-7a-carboxylic Acid Ethyl Ester ((+)-1), and (−)-CPCCOEt, (−)-(1a*S*,7a*S*)-(2-Hydroxyimino-1a,2-dihydro-1*H*-7-oxacyclopropa-[*b*]naphthalene-7a-carboxylic Acid Ethyl Ester ((−)-1). Preparative Chromatographic Resolution. 0.5 g of racemic CPCCOEt was dissolved in 100 mL of hexanes−ethanol 70:30 (v/v). This solution was injected via the pump on a 5-cm × 50-cm Chiralpak AD column[14,15] (Daicel Chemical Industries). The chromatography was achieved at room temperature at a flow rate of 100 mL/min and UV detection was performed at 210 nm. The mobile phase consisted of a mixture of hexanes−ethanol 95:5 (v/v). Under the applied chromatographic conditions, the (+)-enantiomer was isolated from a first fraction collected between 50 and 65 min, and the (−)-enantiomer from a second fraction collected between 80 and 120 min. After a second injection (in total 1.0 g of racemate), 0.473 g of (+)-enantiomer and 0.481 g of (−)-enantiomer, both with an enantiomeric purity greater than 99.9%, were isolated. The enantiomeric purity was determined on an analytical Chiralpak AD column (0.46 × 25 cm); mobile phase, hexanes−ethanol 95:5 (v/v); separation factor, 1.52.

(+)-1: mp 85−86 °C; MS (ES$^+$) $M^+ + 1 = 247.9$; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.35 (t, 3H, J = 7.2 Hz), 1.49 (dd, 1H, J = 5.9 and 7.0 Hz), 2.06 (dd, 1H, J = 5.9 and 11 Hz), 3.4 (dd, 1H, J = 7.1 and 10.6 Hz), 4.31 (dq, 2H, J = 2 and 7.2 Hz), 6.99 (ddd, 1H, J = 1 Hz and 7.1 and 8.1 Hz), 7.04 (dd, 1H, J = 1 and 8.3 Hz), 7.32 (ddd, 1H, J = 1.4 and 7.1 Hz and 8.3 Hz), 7.73 (dd, 1H, J = 1.4 and 8.1 Hz), 8.39 (br s, 1H); ^{13}C

NMR ($CDCl_3$, 50 MHz) δ 14.1, 15.83, 21.1, 61.12, 62.11, 114.42, 117.95, 122.24, 124.3, 131.77, 148.72, 151.45, 169.0; $[\alpha]_D$ = 140.9 (CH_2Cl_2, c = 1); ee = 99.9% (HPLC, Chiralpak AD). Anal. ($C_{13}H_{13}NO_4$) C, H, N.

(−)-1: mp 84−85 °C; MS (ES^+) $M^+ + 1$ = 248.0; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.35 (t, 3H, J = 7.2 Hz), 1.48 (dd, 1H, J = 6.0 and 6.9 Hz), 2.06 (dd, 1H, J = 6.0 and 10.7 Hz), 3.4 (dd, 1H, J = 7.2 and 10.7 Hz), 4.31 (dq, 2H, J = 2 and 7.2 Hz), 6.99 (d, 1H, J = 7.1 and 8.1 Hz), 7.04 (dd, 1H, J = 1 and 8.3 Hz), 7.32 (dd, 1H, J = 7.1 and 8.3 Hz), 7.73 (d, 1H, J = 8.1 Hz) 7.68 (br s, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 14.11, 15.84, 21.16, 61.14, 62.13, 114.43, 117.97, 122.25, 124.29, 131.78, 148.67, 151.46, 169.26; $[\alpha]_D$ = −137.2 (CH_2Cl_2, c = 1); ee = 99.9% (HPLC, Chiralpak AD). Anal. ($C_{13}H_{13}NO_4$) C, H, N.

(+)-CPCCOH, (+)-(1a*R*,7a*R*)-2-Hydroxyimino-1a,2-dihydro-1*H*-7-oxacyclopropa[*b*]naphthalene-7a-carboxylic Acid ((+)-2). A solution of **(+)-1** (200 mg, 0.809 mmol) in THF (20 mL) and aqueous NaOH (0.1 N, 12 mL, 1.2 mmol) was stirred at room temperature. After completion of the hydrolysis (3 h, TLC) the solution was poured into a mixture of brine (100 mL) and EtOAc (35 mL). The aqueous phase was acidified to pH 2 with 1 N HCl and extracted with EtOAc (2 × 35 mL). The combined organic phases were dried (Na_2SO_4) and concentrated in vacuo to afford **(+)-2** (164 mg, 0.75 mmol) in 92% yield: mp 191−193 °C; MS (ES^+) $M^+ + 1$ = 220.0; ^{1}H NMR (CD_3OD, 300 MHz) δ 1.39 (t, 1H, J = 6.5 Hz), 1.97 (dd, 1H, J = 5.9 and 10.6 Hz), 3.31 (m, 1H), 6.96 (m, 2H), 7.3 (br t, 1H, J = 8.6 Hz), 7.75 (br d, 1H, J = 8.3 Hz); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 16.0, 20.8, 61.1, 115.7, 118.2, 122.7, 124.5, 132.0, 146.5, 151.6, 171.0; $[\alpha]_D$ = 146.4 (MeOH, c = 0.58). Anal. ($C_{11}H_9NO_4$) C, H, N.

(−)-CPCCOH, (−)-(1a*S*,7a*S*)-2-Hydroxyimino-1a,2-dihydro-1*H*-7-oxacyclopropa[*b*]naphthalene-7a-carboxylic Acid ((−)-2). (−)-2 was synthesized in analogy to **(+)-2** with a yield of 96%: mp 191−195 °C; MS $M^+ + 1$ = 220.0 (ES^+); ^{1}H NMR (CD_3OD, 300 MHz) δ 1.39 (t, 1H, J = 7.0 Hz), 1.97 (dd, 1H, J = 5.9 and 10.6 Hz), 3.30 (m, 1H), 6.97 (m, 2H), 7.3 (br t, 1H, J = 7.6 Hz), 7.75 (br d, 1H, J = 8.3 Hz); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 16.0, 20.8, 61.1, 115.7, 118.2, 122.7, 124.5, 131.9, 146.5, 151.6, 171.0; $[\alpha]_D$ = −136.5 (MeOH, c = 0.31). Anal. ($C_{11}H_9NO_4$) C, H, N.

(*S*)-2-[((1a*RS*,7a*RS*)-2-Hydroxyimino-1a,2-dihydro-1*H*-7-oxacyclopropa[*b*]naphthalene-7a-carbonyl)amino]-3-phenylpropionic Acid Methyl Ester (3). TBTU (*N*,*N*,*N*′,*N*′-tetramethyl-*O*-(benzotriazol-1-yl)uronium tetrafluoroborate; 140 mg, 0.436 mmol) and ethyldiisopropylamine (0.27 mL, 1.57 mmol) were added to a solution of **(±)-2** (85 mg, 0.38 mmol) in dimethylformamide (2 mL) at room temperature. After 15 min L-phenylalanine methyl ester hydrochloride (100 mg, 0.46 mmol) was added and the reaction mixture was stirred at room temperature. After 2 h, the reaction solution was poured onto a mixture of brine (15 mL) and methyl *tert*-butyl ether (5 mL). The aqueous phase was extracted with methyl *tert*-butyl ether (2 × 5 mL). The combined organic phases were dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by column chromatography (Lobar B, Merck SiO_2, MeOH−CH_2Cl_2 2:98 v/v) yielding 73 mg (50%) of **3** as a colorless glass; a separation of the diastereoisomers was observed using thin-layer chromatography (silica gel, MeOH/CH_2Cl_2 2:98 v/v, eluted twice): R_f (1st diastereoisomer) = 0.73, R_f (2nd diastereoisomer) = 0.6; MS (ES^+) $M^+ + 1$ = 381.0; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.36 (t, 2H, J = 6.5 Hz), 2.04 (dd, 1H, J = 5.9 and 10 Hz), 3.15 (d, 1H, J = 5.3), 3.21 (d, 1H, J = 5.9), 3.4 (m, 1H), 3.71 (s, 1.5 H), 3.76 (s, 1.5 H), 4.96 (m, 1H), 6.7−7.4 (m, 8H), 7.76 (m, 1H), 8.44 (br s, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 14.88, 15.02, 20.21, 38.04, 38.08, 52.38, 53.197, 53.24, 62.90, 115.09, 117.44, 122.53, 122.68, 124.63, 127.21, 128.59, 129.14, 129.25, 131.44, 131.54, 135.75, 135.52, 148.2, 150.9, 168.32, 168.36, 171.57, 171.69; $[\alpha]_D$ = 24.5 (CH_2Cl_2, c = 0.33). Anal. ($C_{21}H_{20}N_2O_5 \cdot 0.19H_2O$) C, H, N.

(*R*)-2-[((1a*RS*,7a*RS*)-2-Hydroxyimino-1a,2-dihydro-1*H*-7-oxacyclopropa[*b*]naphthalene-7a-carbonyl)amino]-3-phenylpropionic Acid Methyl Ester (4). 4 was synthesized in analogy to **3** starting from (±)-2 (185 mg, 0.82 mmol) and D-phenylalanine methyl ester. After purification of the crude material by column chromatography (Lobar B, Merck SiO_2, MeOH−CH_2Cl_2 2:98 v/v) 0.21 g (67%) of **4** was obtained as a colorless oil; as for **3** a separation of the diastereoisomers was observed on TLC (for details see above): MS (ES^+) $M^+ + 1$ = 381.0; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.37 (t, 2H, J = 7.0 Hz), 2.04 (dd, 1H, J = 5.9 and 10.6 Hz), 3.15 (d, 1H, J = 6.4), 3.21 (d, 1H, J = 5.9), 3.33 (m, 1H), 3.72 (s, 1.5 H), 3.76 (s, 1.5 H), 4.95 (m, 1H), 6.7−7.4 (m, 8H), 7.76 (m, 1H), 7.74 (br s, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 14.87, 15.01, 20.19, 38.07, 52.36, 53.19, 53.23, 62.90, 115.10, 115.15, 117.43, 122.51, 124.63, 127.19, 127.22128.58, 129.14, 129.24, 131.43, 131.52, 135.38, 135.52, 148.23, 150.77, 150.90, 168.30, 168.36, 171.55, 171.68; $[\alpha]_D$ = −28.5 (CH_2Cl_2, c = 0.45). Anal. ($C_{21}H_{20}N_2O_5$) C, H, N.

(*R*)-2-[((1a*R*,7a*R*)-2-Hydroxyimino-1a,2-dihydro-1*H*-7-oxacyclopropa[*b*]naphthalene-7a-carbonyl)amino]-3-phenylpropionic Acid Methyl Ester ((+)-5). (+)-5 was synthesized as described for the derivatives **3** and **4** starting from **(+)-2** (164 mg, 0.75 mmol) and D-phenylalanine methyl ester. After purification of the crude material by column chromatography (Lobar B, Merck, SiO_2, MeOH−CH_2Cl_2 0.5:99.5 v/v) 0.081 g (28%) of **(+)-5** was obtained as a colorless glass: MS (ES^+) $M^+ + 1$ = 381.1; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.37 (dd, 1H, J = 5.8 and 7.1 Hz), 2.03 (dd, 1H, J = 5.8 and 10.9 Hz), 3.14 (dd, 1H, J = 6.3 and 13.6 Hz), 3.17 (dd, 1H, J = 5.9 and 13.6 Hz), 3.31 (dd, 1H, J = 7.1 and 10.9), 3.76 (s, 3 H), 4.95 (ddd, 1H, J = 5.9 and 6.3 and 8.3 Hz), 6.92 (dd, 1H, J = 1.1 and 8.2 Hz), 7.02 (ddd, 1H, J = 1.1 and 7.4 and 7.8 Hz), 7.08−7.14 (m, 2H), 7.19−7.28 (m, 3H), 7.33 (ddd, 1H, J = 1.7 and 7.4 and 8.3 Hz), 7.43 (br s, 1H), 7.78 (dd, 1H, J = 1.6 and 7.8 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 15.1, 20.1, 38.2, 52.4, 53.2, 62.9, 115.2, 117.6, 122.7, 124.7, 127.3, 128.7, 129.2, 131.7, 135.5, 148.9, 151, 168.2, 171.6; $[\alpha]_D$ = 45.6 (CH_2Cl_2, c = 0.395). Anal. ($C_{21}H_{20}N_2O_5$) C, H, N.

(*S*)-2-[((1a*S*,7a*S*)-2-Hydroxyimino-1a,2-dihydro-1*H*-7-oxacyclopropa[*b*]naphthalene-7a-carbonyl)amino]-3-phenylpropionic Acid Methyl Ester ((−)-5). (−)-5 was synthesized as described fo the derivatives **3** and **4** starting from **(−)-2** (170 mg, 0.78 mmol) and L-phenylalanine methyl ester. After purification of the crude material by column chromatography (Lobar B, Merck, SiO_2, MeOH−CH_2Cl_2 1:99 v/v) 0.041 g (14%) of **(−)-5** was obtained as a colorless glass: MS (ES^+) $M^+ + 1$ = 381.0; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.37 (dd, 1H, J = 5.9 and 7.0 Hz), 2.03 (dd, 1H, J = 5.8 and 10.6 Hz), 3.14 (dd, 1H, J = 6.2 and 13.6 Hz), 3.17 (dd, 1H, J = 5.9 and 13.6 Hz), 3.31 (dd, 1H, J = 7.0 and 10.6), 3.76 (s, 3 H), 4.95 (ddd, 1H, J = 5.8 and 6.2 and 8.2 Hz), 6.92 (d, 1H, J = 8.2 Hz), 7.02 (dd, 1H, J = 7.4 and 7.8 Hz), 7.08−7.14 (m, 2H), 7.19−7.28 (m, 3H), 7.33 (ddd, 1H, J = 1.7 and 7.4 and 8.3 Hz), 8.02 (dd, 1H, J = 1.6 and 7.8 Hz), 7.43 (br s, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 15.0, 20.1, 38.1, 52.4, 53.2, 62.9, 115.1, 117.5, 122.6, 124.6, 127.2, 128.6, 129.1, 131.6, 135.4, 148.6, 150.9, 168.2, 171.6; $[\alpha]_D$ = −44.3 (CH_2Cl_2, c = 0.6). Anal. ($C_{21}H_{20}N_2O_5$) C, H, N.

(*S*)-2-[((1a*R*,7a*R*)-2-Hydroxyimino-1a,2-dihydro-1*H*-7-oxacyclopropa[*b*]naphthalene-7a-carbonyl)amino]-3-phenylpropionic Acid Methyl Ester ((+)-6). (+)-6 was synthesized in analogy to the derivatives **3** and **4** starting from **(+)-2** (185 mg, 0.82 mmol) and L-phenylalanine methyl ester. After purification of the crude material by column chromatography (SiO_2, MeOH−CH_2Cl_2 2:98 v/v) 0.123 g (38%) of **(+)-6** was obtained as a colorless glass. This material was further chromatographically purified on a reverse-phase column (preparative HPLC Nucleosil 100-7 C18 column, CH_3CN:H_2O 20:80, v/v) and on silica (Lobar B, Merck SiO_2, MeOH−CH_2Cl_2 1:99 v/v) yielding **(+)-6** as a colorless solid which was recrystallized from methyl *tert*-butyl ether. The isolated crystals had a melting point of 138−139 °C: MS (ES^+) $M^+ + 1$ = 381.2; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.37 (dd, 1H, J = 5.9 and 7.0 Hz), 2.04 (dd, 1H, J = 5.9 and 11.0 Hz), 3.20 (s, 1H), 3.22 (s, 1H), 3.33 (dd, 1H, J = 6.9 and 10.9), 3.72 (s, 3 H), 4.95 (dd, 1H, J = 7.6 and 8.2 Hz), 6.88 (d, 1H, J = 8.2), 7.0 (dd, 1H, J = 7.6 and 8.2 Hz), 7.17−7.39 (m, 6H), 7.5 (br s, 1H), 7.75 (d, 1H, J = 7.6 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 14.9, 20.1, 38.2, 52.4,

53.3, 62.9, 115.1, 117.5, 122.6, 124.8, 127.3, 128.7, 129.3, 131.6, 135.6, 148.9, 150.9, 168.2, 171.6; $[\alpha]_D = 108.9$ (CH_2Cl_2, $c = 0.325$). Anal. ($C_{21}H_{20}N_2O_5$) C, H, N.

(*R*)-2-[((1a*S*,7a*S*)-2-Hydroxyimino-1a,2-dihydro-1*H*-7-oxacyclopropa[*b*]naphthalene-7a-carbonyl)amino]-3-phenylpropionic Acid Methyl Ester ((−)-6). (−)-6 was synthesized as described for the derivatives **3** and **4** starting from **(−)-2** (169 mg, 0.77 mmol) and D-phenylalanine methyl ester. After purification of the crude material by column chromatography (SiO_2, MeOH−CH_2Cl_2 1:99 v/v) 0.112 g (38%) of **(−)-6** was obtained as a colorless glass. Recrystallization from methyl *tert*-butyl ether afforded **(−)-6** as colorless crystals: mp 138−139 °C; MS (ES^+) $M^+ + 1 = 381.1$; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.37 (dd, 1H, $J = 5.9$ and 7.0 Hz), 2.04 (dd, 1H, $J = 5.9$ and 11.0 Hz), 3.20 (s, 1H), 3.22 (s, 1H), 3.33 (dd, 1H, $J = 6.9$ and 10.8), 3.72 (s, 3 H), 4.95 (ddd, 1H, $J = 5.9$ and 7.8 and 8.3 Hz), 6.89 (d, 1H, $J = 8.2$), 7.0 (dd, 1H, $J = 7.6$ and 8.2 Hz), 7.15−7.40 (m, 6H), 7.5 (br s, 1H), 7.76 (d, 1H, $J = 7.6$ Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 14.9, 20.1, 38.2, 52.4, 53.3, 62.9, 115.1, 117.5, 122.6, 124.8, 127.3, 128.7, 129.3, 131.6, 135.6, 148.8, 150.9, 168.2, 171.6; $[\alpha]_D = -101.3$ (CH_2Cl_2, $c = 0.43$). Anal. ($C_{21}H_{20}N_2O_5$) C, H, N.

Modeling: Construction of the Model of the TM Domain. The model was built and optimized using the program SYBYL[16] in analogy to other models proposed for GPCRs.[21−23] In brief, seven individual polyalanine standard α-helices of lengths 27, 27, 35, 25, 30, 30 and 24 were built and each was superimposed on the corresponding helix of the α-carbon template derived from the rhodopsin family of GPCRs.[17] The rms distances of superimposed C-α atoms of TM helices I−VII were 0.14, 0.79, 0.19, 0.13, 0.84, 1.35 and 0.12, respectively. All 198 alanines were mutated according to the putative assignment of TM segments given in the SwissProt protein sequence database (see Table 2) by making the corresponding side chain changes of alanines. The length and limits for the helical domains were adjusted to the maximal length of the helices proposed by Baldwin et al.[17] All 10 prolines were fixed using the Sybyl Biopolymer FIX_PROLINE command. Hydrogen atoms were added with the ADDH command and partial charges were computed with the CHARGE GAST_HUCK command. Owing to the presence of charged residues (arginines (5), lysines (9), aspartic acid (1) and glutamic acids (2)) the net charge was +11e. Groups forming termini of helices were kept neutral as NH_2 and COOH. This raw model was optimized with the TRIPOS force field and the X-PLOR[19] software using the conjugate gradient method (TAFF/X-PLOR). Harmonic restraints with a force constant of 1.0 kcal/molÅ^2 were applied to the initial coordinates of all 198 C-α and 188 C-β atoms. Minimization was carried out until the gradient (grad(E)) and the energy (E) were 0.4 kcal/molÅ and −1078 kcal/mol, respectively.

A plausible third extracellular loop between helix VI and VII was searched with SYBYL/BIOPOLYMER/LOOP, assigning residues S808N809 and T815C816 as anchor regions and residues Y810−T814 as a window region. A loop with a sequence homology of 51% and an rms fit of 0.42 for backbone atoms in the anchor regions was selected for building the model, which after automatic construction and calculation of partial charges was again optimized (TAFF/X-PLOR; grad(E) = 0.3 kcal/molÅ, $E = -1089$ kcal/mol) using the same constraints as in the previous minimization.

Docking. Each raw complex obtained by manual docking of the antagonist into the TM domain of the modeled receptor was optimized in 24 independent runs according to an X-PLOR molecular dynamics protocol involving heating from 25 to 500 K in 2 ps and cooling again to 25 K in 2 ps. This was followed by 2000 steps of TAFF/X-PLOR minimization always with harmonic restraints to the previous reference coordinates of the same 198 C-α atoms and a weaker force constant of 0.5 kcal/molÅ^2.

X-ray Crystallography. The X-ray diffraction studies were performed with a Nonius CAD4 diffractometer (Cu Kα radiation; graphite monochromator). The crystals of **(+)-6** were found to be orthorhombic ($P2_12_12_1$) with lattice parameters $a = 10.087(1)$ Å, $b = 10.114(1)$ Å, $c = 38.606(3)$ Å, $Z = 8$. The asymmetric unit contained two crystallographic independent molecules which were conformationally very close and possessed the same configuration on the chromane ring and on the phenylalanine moiety. Further details are given as Supporting Information.

Pharmacology. PI turnover assays in clonal cell lines expressing hmGlu1b were performed as previously described.[13] Each data point represents triplicate measurements expressed as mean ± SEM of at least three independent experiments.

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Supporting Information Available: Coordinate files of Figures 5−7, spatial coordinates of the X-ray structures of **(+)-6**, and elemental analyses. This material is available free of charge via the Internet at http://pubs.acs.org.

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